COMPUTATIONAL MODELING OF OPTOGENETIC SWITCHING OF NEURONS

A synopsis of the proposed thesis to be submitted for the degree of

DOCTOR OF PHILOSOPHY

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September, 2015

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1. INTRODUCTION

The human brain is the most intricate organ in the body and has been referred to as "The most complex object in the known universe". A recent estimate puts the number of neurons in the human brain at 86 billion with each neuron forming hundreds, if not thousands of individual connections through which communication takes place at millisecond time scale. The complexity of its structure and function is astounding. For neuroscientists, this makes the brain both irresistibly fascinating and prohibitively difficult to study. In order to overcome the challenges that result from the inherent complexity of the brain, investigators have to be creative and innovative in their approach to examine it [Williams et al. (2015)].

Each cell in the large network of interconnected neurons in the brain functions as a nonlinear processing element. Unraveling the mysteries of information processing in the complex networks of the brain requires versatile neurostimulation and imaging techniques [Deisseroth et al. (2010)].

Over the last few decades, the dominant hypothesis in treating neurological and psychiatric diseases has been the *chemical imbalance paradigm,* i.e., assumes that any mental disorder is the result of imbalance in the concentration of chemicals in the central or peripheral nervous system. Based on this hypothesis, such diseases can be curable if we invent mechanisms to control and monitor the concentration of the corresponding chemicals, by using appropriate pharmacological substances.

Although, this approach has been partially successful in treating several mental disorders such as depression or anhedonia that are caused by the reduction in the concentration of the monoamine neurotransmitter serotonin [Levinson et al. (2006)], recent advances in the development of brain interface instrumentation and prosthetic devices has opened a new line of research to treat mental diseases by speaking the electrical language of neurons, usually known as *interventional psychiatry*. *Deep-brain stimulation* (DBS) is an example of this approach, which has been reasonably successful in treating some neurological diseases including Parkinson's [Chou et al. (2012)]. Nonetheless, most interventional therapeutic procedures lack specific-cell-type targeting capabilities and potentially cause serious side effects. For instance, implanted electrodes in Parkinsonian patients stimulate most cells in their reach with no preference to target any specific cell-type, and as a result, cause several side effects including depression, mood alteration, or sensory and motor control problems which are all suppressed by turning off the stimulations pulses.

To address this challenge, a new neurostimulation technique, known as optogenetics, has been invented that combines the tools of molecular genetics with recent advances in the fields of optics and photonics [Boyden et al. (2005), Deisseroth et al. (2006), Nagel et al. (2005), Han et al. (2007), Han et al. (2009), Chow et al. (2010), Miesenbock et al. (2011), Laun et al. (2014), Grosnick et al.

(2015)]. In this technique, a family of light-gated microbial opsin proteins that function as light-activated proteins are expressed in genetically targeted neurons [Zhang et al. (2007)], Gradinaru et al. (2008)]. Once these light sensitive proteins are expressed in a neuron, the activity of the cell can be increased or suppressed, with millisecond temporal accuracy, by exposing the cell to light with appropriate wavelengths, even without the addition of the exogeneous cofactor in mammalian cells. [Paschaie et al. (2014)]

Optogenetics, the use of light-sensitive ion channels for stimulation of mammalian cells and tissues, offers specificity and superior precision of control compared to traditional chemical or electrical means of stimulation. The development of new optogenetic tools that allow for more efficient optical stimulation can be guided by computational prediction of the response of different cells and tissues to light.

Some major advantages of optogenetic stimulation are the following :

1) Specific cell-type targeting can be achieved in optogenetics, by controlling the gene delivery process to express light sensitive proteins predominantly in the cell-type of interest, by using appropriate promoters [Gradinaru et al. (2007)].

2) Bidirectional control of cellular activities is feasible in optogenetics. It is possible to simultaneously express cation channels and anion pumps that are sensitive to different wavelengths in one cell-type of interest. Exposing the cell to appropriate wavelengths, results in excitation and inhibition respectively [Zhang et al. (2007)], Mattis et al. (2011)].

3) The inherent parallelism of optics can help to manipulate neural activities in large-scale neural networks of the brain, particularly in the cortex.

Some major applications of optogenetic neuromodulation are the following:

1. ***Cracking Neural Codes:*** Neurons communicate by generating sequences of action potentials while information is embedded in the *mean firing rate* or the *precise timing* of action potentials. Since, optogenetic tools provide a bidirectional mechanism to control neural activities with millisecond temporal resolution, engineering a sequence of light pulses that can represent a specific neural code can produce any firing pattern. With a suitable quantitative readout, the effect of the generated codeword on the postsynaptic neurons or the corresponding behavioral phenotypes can then be investigated.

2. ***Interrogating Neural Circuits:*** Optogenetics enables the dissection of mental disease circuits by providing a discriminative mechanism for controlling the activity of cell-types in a neural network By providing a discriminative mechanism for controlling the activity of cell-types in a

neural network (e.g., Parkinsonian neural circuits or interrogation of the role of circuit elements in the overall dynamics of the network). [Gradinaru et al. (2009), Sohal et al. (2009)].

3. ***Generating Reversible Models of Neurological Diseases:*** The mechanism for bidirectional control of genetically designated neural populations gives the opportunity to emulate and study new models of neurological diseases (e.g., optogenetics can be used to reversibly generate patterns of epileptogenic activity [Zhang et al. (2007)]).
4. ***Developing New and Efficient Therapeutic Treatments:*** Optical modulation of activity in targeted cell-types can help in the discovery of efficient treatments for mental diseases with minimum side effects, since we can selectively modulate the activity of excitatory or inhibitory neurons separately (e.g., hyperpolarization of glutamatergic neurons in subthalamic nucleus with expression of NpHR and suitable light exposure has shown to be effective in treating Parkinson's in animal models of the disease) [Gradinaru et al. (2009)].

The advent of optogenetics has opened new lines of research to develop other light sensitive ion channels with faster or slower kinetics or different spectral sensitivities. It also opens new fields of investigation to control the distribution of light inside the three-dimensional structure of the brain tissue or combine optogenetics with other stimulation or imaging modalities including electrophysiology, electrocorticography (ECoG), nonlinear microscopy, and functional magnetic resonance imaging [Paschaie et al. (2014)].

Optogenetic experiments require suitable light delivery instrumentation that has small physical size, stability, intensity tunable and low cost. The three common types of light sources are the arc lamp, laser and more recently the LED. Initially, light stimulation in the neuron was achieved by guiding light in optical fiber electrode implanted in the brain. However the requirement of compact and light-weight power source has been fulfilled with the successful implantation of LED in brain. The implanted LED remains effective over 50 days after surgery, which shows long term stability of the light source [Rossi et al. (2015)]. This provides a great opportunity for wireless optogenetics. A 20 mg, 10 mm dimension, wireless optogenetic device which includes a power-receiving coil, IC and LED is easy to implant not only in brain but spine or limbs also with minimal tissue heating ($< 1^{o}C$) [Montgomery et al. (2015].

1.1. Light-Activated Proteins used in Optogenetics

Optogenetics applies light-sensitive proteins, which have been isolated from various microorganisms and plants, to manipulate excitable cells in heterologous systems (Miesenbock et al. (2011)]. Initial

work in the field used naturally occurring photosensitive proteins such as channelrhodopsin (ChR) [Boyden et al. (2005)] and halorhodopsin (HR) [Zhang et al. (2007)] to induce activation or inhibition of neural activity in mammalian neurons respectively. This has opened up a rapidly expanding field utilizing additional genetically encoded actuators in a wide range of applications [Fenno et al. (2011)]. Beyond the utilization of such naturally-occurring photoreceptors, protein engineering over the last decade has generated an expanded optogenetic toolbox for more precise and effective manipulation, allowing refined modes of control that are tailored to individual systems. The most widely used optogenetic tools are proteins of the microbial rhodopsin family [Paschaie et al. (2014)].

Microbial rhodopsins are single-component units, which can transform light energy into current with efficiency higher than any man-made mechanisms so far. Microbial rhodopsins such as Bacteriorhodopsin (BR) and Halorhodopsin (HR) are found in the archaea *Halobacterium salinarium,* which inhabit extreme conditions in salt marshes. [Bodaker et al. (2012)].

In these ancient halobacteria, rhodopsins use light energy for generating the electrochemical gradient required for ATP synthesis, in the absence of oxygen and for withstanding the high osmotic pressure [Olbe et al. (2000)]. In 2002, a new member of the microbial rhodposin family called Channelrhodopsin (ChR), was isolated from the fresh water algae *Chlamydomonas rheinhardtii* [Nagel et al. (2002)]. Unlike previously discovered microbial rhodopsins, which typically serve as ion pumps and transport ions against electrochemical gradients, ChRs possess an aqueous ion channel pore, allowing passive conduction of cations across the cell membrane, according to the electrochemical gradient [Nagel et al. (2003)]. Therefore, microbial rhodopsins can be grouped into pumps, acting as photodiodes, and light-triggered ion-channels, which can be considered as light-dependent resistors.

All microbial rhodopsins rely on the same fundamental photoreaction comprising the isomerization of a covalently bound retinal cofactor, regardless of the mechanism of action or the ion species conducted. Upon photon absorption, the cofactor isomerizes from the *all-trans* to *13-cis* conformation. This conformational shift changes the dipole moment of the retinal molecule and initiates pronounced structural rearrangements of the protein, which finally lead to the transport of ions [Zhang et al. (2011)]. All these complex rearrangements occur within less than a millisecond, whereby the activated rhodopsin cycles through various photochemically distinct states.

Channelrhodopsin-2 (ChR2) is the first light-sensitive ion-channel that after its cloning in 2003 [Nagel et al. (2003)], found widespread use as an optical actuator in neuroscience [Boyden et al. (2005), Yizhar et al. (2011)], and more recently in cardiac applications [Abilez et al. (2011)]. ChR2 is both light and voltage-sensitive. The conducting pore of the channel associates (via a covalent bond) to

retinal, which serves as the chromophore (the light-sensing element). Interaction of all-trans retinal with a photon of the proper wavelength (470 nm) leads to instantaneous isomerization to *13-cis*-retinal. This transition triggers the opening of the ion channel allowing cation movements down their electrochemical gradient, with preferential selectivity to H^+ [Nagel et al. (2003), Lin et al. (2009)]. ChR2 provides exclusively inward current (at negative membrane potentials) with a reversal potential near 0 mV. The single channel conductance for the wild type ChR2 is small compared to classical excitatory ion channels (e.g. sodium channels) with reported values ranging from 40–90 fS [Nagel et al. (2003), Feldbauer et al. (2009)] to 0.25–2.42 pS [Lin et al. (2009)]. Genetically engineered mutants of ChR2, e.g. H134R [Nagel et al. (2005)], T159C and ET/TC [Berndt et al. (2011)], offer augmented excitatory current; while other mutants alter kinetics to serve different uses, e.g. fast ChETA [Gunaydin et al.(2010)] or stable switches with a prolonged open state [Berndt et al. (2009)]. As a prototypical opsin, ChR2 has become the focus of the initial efforts to create a computational branch of optogenetics [Williams et al. (2013)].

This series of states can be collectively described as a photocycle in various levels of detail. A four-state electrophysiological photocycle, explains the major electrical features of channel function, including inactivation, photocurrent kinetics, and adaption phenomena as observed in electrical recordings from cells expressing ChR [Stehfest et al. (2010)]. For example, a fully dark adapted ChR2 molecule activated with a short blue light pulse first proceeds through an open state of high conductance (O1), causing a transient peak photocurrent and then enters a lower conductive state (O2) which leads to lower photocurrent. This drop in photocurrent is commonly referred as inactivation, and can be as large as 72% of the initial current for the wild-type ChR2 [Prigge et al. (2012)]. The lifetime for the conductive states is estimated to be 10 ms [Feldbauer et al. (2009)]. Molecules in the open state can transition to two different dark states with an apparent τ_1, τ_2 of 21 ms [Prigge et al. (2012)], Feldbauer et al. (2009)].

Upon repeated illumination at this stage, molecules reexcited from the second dark state pass through the low conductance state, giving rise to a light-adapted steady-state photocurrent, which is smaller than the initial peak current. Consequently, a series of 2-ms light pulses will cause attenuation of the photocurrent to a lower equilibrium level [Prigge et al (2012)]. Only after a recovery period of > 6 s, all molecules will have returned to their initial dark-adapted state.

To achieve light-based control of neural function, these bioelectric units have been selectively targeted to different excitable cells such as neurons, heart, or muscle cells [Boyden et al. (2005), Bruegmann et al. (2010)]. In neurons, microbial pumps such as HR or BR induce a square-like hyperpolarization response of the membrane potential upon illumination [Zhang et al. (2007)], which can rapidly and effectively silence neural activity. ChRs perform the opposite function of

depolarizing neurons in response to illumination by allowing positively charged cations to passively enter the cell. ChRs therefore serve as light-induced activators and pulse activation of neurons expressing ChR2. Since, light naturally penetrates intact biological tissues, the optogenetic approach has been extended to living organisms, including nematodes, flies, zebrafish, rodents, and monkeys [Nagel et al. (2005), Douglass et al. (2008)]. Driven by the success of the initial approach with microbial rhodopsins isolated from several species, large genomic screens for new microbial rhodopsins in all kingdoms of life have led to the discovery of several new variants, which show diverse functional properties such as spectral tuning, ionic selectivity, and compatibility towards mammalian cell expression [Govorunova et al. (2013)].

These proteins, encoded by genes from diverse microbial species, were subjected to molecular engineering, which aimed to increase their efficacy as light-driven tools for controlling neural excitability. Generally, two lines of improvement strategies have been applied. The first is aimed at increasing the biocompatibility to mammalian cells, while the second approach is to modify the intrinsic properties of the actuator along one or more functional dimension.

The most commonly used tools for neural silencing are the two enhanced versions: NpHR3.0 and Arch3.0 [Mattis et al. (2011)], whereas the main improvements for hyperpolarizing tools were focused on increasing photocurrent amplitude through enhanced membrane targeting.

Molecular engineering of ChR variants has yielded numerous tools with distinct spectral and kinetic properties. Two mutations, H134R and T159C, have been shown to increase photocurrent amplitude in pyramidal neurons by a factor of 1.5 to 2 [Nagel et al. (2005), Berndt et al. (2011)]. In both variants, this change corresponds with slower off-kinetics of 30–60 ms in neurons corresponding to a prolonged life-time [Mattis et al. (2011), Prigge et al. (2012), Felldbauer et al. (2009)].

One of the key amino acids controlling the protonation state of the retinal chromophore is a glutamate at position 123 in the ChR2 sequence. Substitution of an alanine or threonine at this position generated the E123A/T mutants, in which the photocycle is accelerated to an apparent off-kinetics of 4–6 ms [Gunaydin et al. (2010)]. These mutations can increase the fidelity of high frequency evoked action potential trains. However, since the open state is populated only for shorter times, the amount of ions conducted during this interval is decreased. This is effectively manifested in reduced photocurrent amplitude. For the same reason, the necessary light intensity to reach half saturation is increased from 1 mW/mm^2 to 5 mW/mm^2 [Mattis et al. (2011)], placing constraints over *in vivo* applications in which tissue heating could in fact pose a significant challenge [Cardin et al.(2010)].

To address the heating issue and to increase the effective light sensitivity of optogenetic excitation, light switchable variants have been engineered. Mutations at positions C128 and D156 can substantially delay the closing reaction of the pore. The single mutations C128S and D156A slow down the apparent off-kinetics of ChR2 to 120 and 250s respectively [Berndt et al. (2009)]. Combining both mutations generated a variant with a closing time constant of 30 min. Spectroscopic data has revealed that as with the wild-type ChR2 [Bamann et al. (2008)], yellow light can facilitate the closing reaction [Yizhar et al. (2011)]. Hence, these mutants can be triggered into the open state by a brief blue light pulse and can then be flashed back to the closed, dark state with intense yellow light. Due to the of–on nature of the light-driven changes in membrane potential, these tools have been named step function opsins (SFOs) [Berndt et al.(2009)]. Since activated SFOs accumulate in the open state for the entire duration of blue light illumination, even low light intensities will render all molecules active when pulse duration is prolonged. Therefore, those variants act as photon integrators and are thus 100 times more light-sensitive than naturally occurring microbial rhodopsins [Yizhar et al. (2011)]. Long low-intensity light pulses can also reduce the variability of activation throughout the targeted cell population *in vivo*, since the full population of opsin-expressing cells, even in a large volume of tissue, could depolarize over time [Yizhar et al. (2011)]. With respect to ion selectivity, under physiological ionic conditions and membrane resting potential of a neuron, the photocurrent of ChRs is composed of 65% H^+, 35% Na^+, and 1% Ca^{+2} and Mg^{+2} [Schneider et al. (2013)].

Only few mutations have been shown to have a significant impact on ion selectivity. Most notably the L132C mutation in ChR2, was found to increase conductance of the divalent cations Ca^{+2} and Mg^{+2} by a factor of five [Schneider et al. (2013)]. The ChR2 L132C/T159C mutants show large photocurrents with an increased conductance of divalent cations. Since Ca^{+2} can trigger intracellular signaling cascades, these variants can potentially be used to manipulate cell activity by changing intracellular ionic composition. Such alterations to the ionic selectivity of ChRs can potentially be very useful, if selectivity can be manipulated to the extent that only single ions (e.g., sodium, potassium, or chloride) can be exclusively conducted. Although this is probably a difficult task, it is likely that the recently resolved 3D crystal structure of ChR will further boost this type of molecular development [Kato et al. (2015)].

Following the success of applying microbial rhodopsins as optogenetic tools in mammalian neurons, similar approaches have been applied to engineer a range of light-driven actuators that allow the manipulation of diverse cellular processes. One common approach involves addition or substitution of enzyme domains with photoreceptor units, which exhibit structural changes upon illumination. For this purpose, the most commonly used photoreceptor domains are LOV (Light-Oxygen-Voltage

domain), BLUF (Blue Light Sensor using FAD), cryptochromes and phyotchromes. These photoreceptors all bind to organic cofactors such as flavins and different porphyrin molecules which essentially perform a similar role to that played by the retinal chromophore in the rhodopsins [Oglich et al. (2010)]. Here, most notable is a photoactivatable adenylate cyclase bPac, which was found in the bacteria *Beggiatoa sp. Habitas* hydrogen sulfide rich environments. This cyclase is light-sensitive via a BLUF domain. In Drosophila, bPac expression increases the level of signaling molecule cAMP by a factor of ten upon blue light illumination [Stierl et al. (2011)].

Recently, the toolbox was extended by a very promising member a light-inducible transcriptional effector for endogenous gene expression. Here, a light-inducible dimerization of a cryptochrome called Cry2 with its native truncated binding partner CIBN [Kennedy et al. (2010)], was fused to a transcription effector domain and a DNA recognition domain, respectively. Upon blue light illumination, the DNA bound CIBN recruits the Cry2-fused activator domain and can thereby increase the transcription of target genes [Konarmann et al. (2013)]. These and other tools have extended the range of optogenetic manipulation beyond the simple control of neural excitability and are likely to contribute significantly to a range of experimental applications [Paschaie et al. (2014)].

1.2. Computational Optogenetics

Quantitative biophysical approaches have strong traditions in neural and cardiac electrophysiology [Noble et al. (2002), Kleber et al. (2004), Hodgkin et al. (1952)]. The Hodgkin and Huxley's empirical modeling framework is still quite successful in explain the dynamic and highly nonlinear process of cellular excitation [Hodgkin et al. (1952)], even though, the complexity of computational models is ever increasing to reflect new processes such as the diversity in ion-channels and their characteristics. The function of classical ion channels is described by voltage and time dependent equations. In some cases, additional chemical or mechanical control parameters are included. Only recently, light sensitive ion channels originally found in bacteria or yeast has proven relevant to mammalian electrophysiology [Boyden et al. (2005), Nagel et al. (2005)]. These ion channels provide alternative (optical) means of excitation with superior (cell type) specificity and spatio-temporal resolution compared to electrical stimulation.

Computational optogenetics, i.e. quantitative modeling for virtual experimentation in optogenetics, is in its infancy with only a handful of publications [Hegemann et al. (2005), Golomb et al. (2006), Nikolic et al. (2009), Talathi et al. (2010), Abilez et al. (2011), Grossmann et al. (2011b), Grossman et al. (2013), Nikolic et al., (2013), Stefanescu et al. (2013), Williams et al. (2013), Stujenske et al. (2015)]. It can aid in quick (virtual) testing of the behavior of newly developed tools in a wide range of cell types, and within realistic tissue settings. Such simulations can also drive the rational design of new optogenetic tools optimized for a specific cell/tissue environment. Furthermore, they can

assist in the proper interpretation of experiments within a complex cell/tissue environment, which is often ambiguous and challenging.

ChR2's photocycle has been studied extensively and it formed the basis for abstracting the behavior of the channel to Markov type (three- and four-) state models proposed recently by Hegemann and Nikolic [Hegemann et al. (2005)] and Nikolic et al. (2009)]. Versions of a four-state model are currently favored based on experimental evidence for four kinetic intermediates with differing time constants [Hegemann et al. (2005), Stehfest et al. (2010)]. Recently a six-state kinetic analysis of ChR2 has also been carried out for better understanding of the process [Grossmann et al. (2013), Nikolic et al. (2013)].

Current efforts also include modeling of different mutants [Grossmann et al. (2011)], as well as the integration of ChR2 into comprehensive cell models – neurons [Grossmann et al. (2011), Talathi et al.(2010), Foutz et al. (2012)] and cardiomyocytes [Abilez et al. (2011), Wong. et al.(2012)]. While ChR2's light sensitivity has been studied extensively [Nagel et al. (2003)], data on its voltage dependence is limited [Chater et al. (2010)]. Early models assumed a linear current-voltage relationship [Nikolic et al. (2009), Talathi et al. (2010)]. More recent ChR2 model have incorporated some nonlinearity as experimental results demonstrate prominent inward rectification [Nagel et al. (2005), Lin et al. (2009), Chater et al. (2010), Grossmann et al. (2011), Abilez et al. (2011)]. Voltage sensitivity, however, has yet to be comprehensively addressed. Proper quantitative prediction of the functionality of light sensitive ion channels, including ChR2, within mammalian cells and tissues, requires that the kinetics and amplitude of the observed current are scaled to reflect physiological conditions, even when the channel is characterized primarily at room temperature. In a recent study, a rigorously validated quantitative model of H134R, a mutant of ChR2 with enhanced current has been proposed. The H134R variant has been studied based on experimental data collected over a wide range of irradiances and voltages. The result is a new ChR2 model with updated voltage dependences not only of the current-voltage response, but also of relevant kinetic parameters and scaling factors (Q10) to adjust processes to physiological temperature [Williams et al. (2013)].

1.3. Challenges

Despite the increased number of applications, all present optogenetic tools utilize light in the visible range. New tools, sensitive at the longer range of the electromagnetic spectrum (e.g., infrared or radio frequency), are desirable since they would allow deeper tissue penetration and perhaps even facilitate non-invasive approaches. Promising candidates for this approach, for example, are the infrared thermal sensitive ion-channels from bats and phythochrome-based tools [Gracheva et al. (2011), Gracheva et al. (2010)].

Tools which can be specifically switched ON and OFF with minimal cross excitation would allow consecutive control of different cellular processes, without optically cross-activating each other in the same organism. General speaking, ChRs impose another activation pathway on top of naturally occurring ones.

Upon light stimulation, action potentials can be evoked in an all-or-none fashion. From an analytical point of view, genetically-encoded tools rendering specific endogenous light activatable proteins would be more valuable, since they would enable researchers to modulate the endogenous cellular mechanisms which drive action potentials. But the greatest need for improvements in the field of optogenetics is the development of cellular activity reporters. Particularly, fast, noninvasive reporters for membrane potential at the far end of the electromagnetic spectrum would be very valuable.

In addition, effective optogenetic reporters at different wavelength for Ca^{+2}, cAMP, cGCMP, or different neuromodulators such as dopamine or acetylcholine are still needed or have room for improvement. Together with the growing range of optogenetic actuators, these tools will allow the characterization of brain dynamics on levels other than electrical activity. Manipulating cellular processes with light at high spatial and temporal resolution has already been proven as an indispensable method in neuroscience research. Therefore, the pre-paradigmatic stage, where optogenetic tools are developed just in a proof-of-concept fashion has passed. With the increasing knowledge of the mechanisms of action of these proteins and the complexities of their application in neural systems, new tools need to be engineered such that they maximally fulfill their intended function and allow predictable, well-controlled manipulation of neural activity [Pashaie et al. (2014)].

2. MOTIVATION

Computational optogenetics can aid in quick (virtual) testing of the behavior of newly developed tools in a wide range of cell types, and within realistic tissue settings. Such simulations can also drive the rational design of new optogenetics tools optimized for a specific cell/tissue environment [Williams et al. (2013)].

There is tremendous scope for research in computational optogenetics. New ChR mutants have come to light that include, red-shifted mutants (C1V1, C2V2, Chrimson, Chronos, CoChR) [Klapoetke et al. (2014)] and blue-shifted mutants (E169Q, D299N, which show μs kinetics at μW power) [Spudich et al. (2015), Kato et al. (2015)], Arch Mac [Chow et al. (2010)], LOV domain and BLUF [Bergeijk et al. (2015), Pudasaini et al. (2015)]. A new class of channelrhodopsin converted into chloride – conducting anion channels (ChloC), have been designed to enable fast optical inhibition of action potentials that can also be engineered to display step-function kinetics for stable inhibition [Berndt et al (2014), Weitek et al. (2014)].

Some ChR mutants are activated and inactivated by different wavelengths for eg. C128 is activated at 470 nm and deactivated at 560 nm. D156A is activated at 470 nm and deactivated at 590 nm. Combination of both mutations C128/ D156 also shows activation and deactivation at 445 nm and 590 nm respectively. These mutants sustain bistable function as they have large membrane time constant (τ > 20ms.). For C128A, τ ~52 s, for C128S, τ ~106 s [Bernt et al. (2009)], D156A τ ~ 300 s [Banmann et al. (2008)]. New bistable variants C128T, C167 and D195 have been reported recently [Ritter et al. (2013), Shoko Hososhima et al. (2015)]. The deactivation processes of these bistable variants are dependent upon light in contrast to ChR in which deactivation was a spontaneous process. There is no kinetic model to describe the bistable operation as yet.

The efficiency of neural stimulation with ChR2 drops at high frequencies, often before the natural limit of the neuron is reached. This drop in efficiency at high frequencies led to efforts to develop fast mutants of ChR2, namely, ChETA, ChD, CatCh and ChRET/TC [Grossman et al. (2011a), Stefanescu et al. (2013)]. ChETA reduces extra spikes, eliminates plateau potentials and allows temporally stationary sustains spike trains upto at least 200 Hz [Gunaydin et al. (2010]. Another important approach would be to theoretically determine optimized photoresponse of these photosensitive proteins for optimal neural spiking. It would also be interesting to investigate the design of neuromorphic devices for computing applications.

3. PROPOSED RESEARCH OBJECTIVES

It is proposed to undertake a detailed theoretical study of new photosensitive proteins used in optogenetics, to control the switching action of neurons. It is proposed to investigate optimized conditions with respect to light such as intensity, concentration, wavelength, frequency and pulse width.

The specific objectives of the study are outlined as follows:

1. It is proposed to undertake a detailed analysis of accurate kinetic models incorporating various intermediate states in the photocycles of different newly discovered photosensitive proteins, for both excitation and inhibition, such as blue-shifted ChR's Mutants (E169Q, D299N), red-shifted ChR's Mutants (C1V1, C2V2, Chemeric), and Arch, Mac proteins respectively.
2. It is proposed to study the effect of various parameters such as Intensity, wavelength, concentration, frequency and pulse width of illumination on neural switching.
3. It is proposed to formulate an accurate model for the bistable operation of ChR's mutants.
4. It is proposed to analyze high frequency response of optogenetic neural switching and investigate conditions for ultrafast operation.
5. It is also proposed to investigate the possibility of designing neuromorphic devices for computing applications.

4. WORK ALREADY DONE

An extensive literature survey has been carried out. Kinetic models available for natural photosensitive proteins such as ChR, ChR Mutants (ChRwt, ChRET/TC, ChETA), NpHR have been studied. Results reported in literature for 3 and 4 state models of ChR and ChR mutants have been reproduced [Stefanescu et al. (2013)].

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